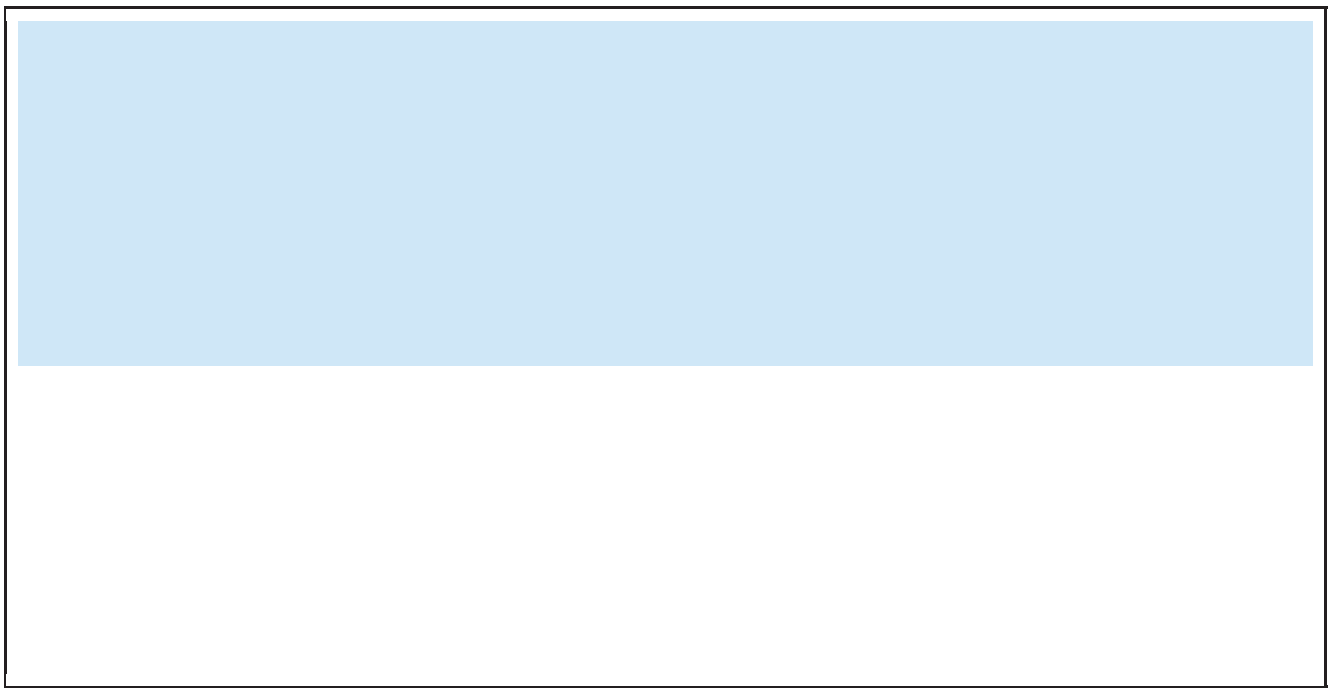
Lack of p53 Affects the Expression of Several Brain Mitochondrial Proteins: Insights from Proteomics into Important Pathways Regulated by p53

Ada Fi i i[1,2], R h a a S a a[2], E ge i Ba e[1,3], Gi a a Ce i i[2]



Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

2D-PAGE was performed to separate proteins on IEF strips based on molecular migration rate. IEF strips were thawed and equilibrated for 10 min in equilibration buffer A [50 mM Tris HCl, pH 6.8, 6 M urea, 1% (w/v) SDS, 30% v/v glycerol, 0.5% DTT] and then re-equilibrated for 10 min in equilibration buffer B [50 mM Tris HCl, pH 6.8, 6 M urea, 1% (w/v) SDS, 30% v/v glycerol, 4.5% IA]. Criterion precast linear gradient (8 16%) Tris HCl polyacrylamide gels were uesd to perform second dimension electrophoresis. Precision Plus Protein™ All Blue Standards and samples were run at a constant voltage of 200 V for 65 min.

SYPRO RubyH staining

After 2D-PAGE, gels were incubated in a fixing solution [7% (v/v) acetic acid, 10% (v/v) methanol] for 20 min at RT. Sypro RubyH Protein Gel Stain, (50 ml) was added to gels to stain them overnight at RT on a gently rocking platform. Gels then were placed in deionized water at RT until scanning. Gels were scanned into Adobe Photoshop 6.0 with a Molecular Dynamics STORM Phosphoimager (lex/l em: 470/618 nm) and stored in deionized water at 4uC until further use.

Image Analysis

for Differential expression. Spot intensities from SYPRO Ruby-H-stained 2D-gell-sttensities from

Intensities were normalized to total gel densities and/or densities of all valid spots on the gels. Only spots with a 1.5-fold increase or decrease in normalized spot density in those samples and a statistically significant difference based on a Student's *t*-test at 95% confidence (i.e. $p < 0.05$) were considered for MS/MS analysis.

In-gel trypsin digestion

In-gel trypsin digestion of selected gel spots was performed as previously described [23]. Briefly, protein spots identified as significantly altered were excised from 2D-gels with a clean, sterilized blade and transferred to Eppendorf microcentrifuge tubes. Gel plugs were then washed with 0.1 M ammonium bicarbonate (NH_4HCO_3) at RT for 15 min, followed by incubation with 100% acetonitrile at RT for 15 min. After solvent removal, gel plugs were dried in their respective tubes under a flow hood at RT. Plugs were incubated for 45 min in 20 µL of 20 mM DTT in 0.1 M NH_4HCO_3 at 56°C. The DTT/NH_4HCO_3 solution was then removed and replaced with 20 µL of 55 mM iodoacetate (IA) solution in 0.1 M NH_4HCO_3 and incubated with gentle agitation at room temperature in the dark for 30 min. Excess IA solution was removed and plugs incubated for 15 min with 200 µ

The identified proteins were: guanine nucleotide-binding protein G (o) subunit alpha (212-fold p53KO, *P<0.0019), ATP synthase subunit beta (125-fold p53KO, *P<0.0035), heat shock cognate 71 (212-fold p53KO, *P<0.002), aldehyde

prevent the formation of amyloid fibrils [48], and previously, HSC-71 was found down regulated [49], and oxidatively modified in AD brain [50]. Therefore the increase of HSC-71 expression levels, induced by the lack of p53, conceivably could play a protective role in AD progression.

Energy dysfunction and mitochondrial alterations

Several findings suggest that p53 has a role in the regulation of pathways involved in glucose metabolism, supporting oxidative phosphorylation and the pentose phosphate shunt, and inhibiting glycolysis [11]. These activities of p53 prevent cancer development. In addition, mitochondria are a major site in which some constituents of these pathways play a role. Therefore, there is a connection between p53 and mitochondria [51], and a better understanding of this link conceivably could provide insight into the progression of mitochondria related disorders.

In our study VDAC was found up-regulated in mitochondria of $p53^{(-/-)}$ mice compared to mitochondria from WT mice. VDAC is a component of the mitochondria permeability transition pore (MPT), which allows the exchange of metabolities like ATP in and out of mitochondria, and it is also involved in synaptic communication and in the early phases of apoptosis [52]. Previous studies revealed the anti-apoptotic function of VDAC through its ability to bind BAK, a pro-apoptotic protein [53]. Likewise, VDAC may restrain p53, reducing its levels [54]. Therefore, these prior results suggest that VDAC and p53 are interconnected, and that lack of p53 could increase the expression of VDAC, in according with our results. The upregulation of VDAC conceivably could improve synaptic transmission and cell survival as well as modulate apoptotic events.

In addition, in our study we found several energy-related proteins: ATP synthase subunit beta, mitochondrial isoform of fumarate hydratase, and cytochrome bc1 complex Rieske subunit, over-expressed in brain mitochondrial of $p53^{(-/-)}$ mice. Since inhibition of p53 leads to dependence of cells on glycolysis and to considerable impairment of aerobic pathways [55], our data may reflect a stress response to compensate for this effect. Moreover the p53-dependent protein targets may be highly cellular type specific. Accordingly, our results also may reflect the high glycolytic metabolism in brain. The over-expression of these proteins, involved in energy metabolism, seems to confirm the hypothesis of this work, in which diminution of p53 may represent a target to restore mitochondrial dysfunction, since these proteins were found altered in models of aging and neurodegenerative diseases [56 58].

p53 plays an additional role in the regulation of glutamate metabolism activating the expression of glutaminase 2 which provides glutamate to promote the tricarboxylic acid (TCA) cycle and oxidative phosphorylation [59]. Glutamate may be oxidatively

9. Butterfield DA, Reed T, Newman SF, Sultana R (2007) Roles of amyloid β-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer s disease and mild cognitive impairment. Free Radical Biology and Medicine 43: 658 677.
10. Momand J, Wu H-H, Dasgupta G (2000) MDM2 master regulator of the p53 tumor suppressor protein. Gene 242: 15 29.
11. Liu B, Chen Y, St. Clair DK (2008) ROS and p53: A versatile partnership. Free Radical Biology and Medicine 44: 1529 1535.
12. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B (1997) A model for p53-induced apoptosis. Nature 389: 300 305.
13. Johnson TM, Yu ZX, Ferrans VJ, Lowenstein RA, Finkel T (1996) Reactive oxygen species are downstream mediators of p53-dependent apoptosis. Proc Natl Acad Sci U S A 93: 11848 11852.

66. Murphy TC, Amarnath V, Picklo Sr MJ (2003) Mitochondrial oxidation of 4-hydroxy-2-nonenal in rat cerebral cortex. Journal of Neurochemistry 84: 1313 1321.
67. Butterfield DA, Bader Lange ML, Sultana R (2010) Involvements of the lipid peroxidation product, HNE, in the pathogenesis and progression of Alzheimer s disease. Biochimica et Biophysica Acta (BBA) Molecular and Cell Biology of Lipids 1801: 924 929.
68. Yoon K-A, Nakamura Y, Arakawa H (2004) Identification of ALDH4 as a p53-inducible gene and its protective role in cellular stresses. J Hum Genet 49: 134 140.
69. Papazoglu C, Mills AA (2007) p53: at the crossroad between cancer and ageing. The Journal of Pathology 211: 124 133.